**REMARKS**

In view of the following Remarks, the Examiner is requested to withdraw the rejection and allow Claims 1-8, 13, 17, and 27, the only claims pending and currently under examination in this application.

**FORMAL MATTERS:**

Claim 12 is canceled without prejudice.

Claims 1 is amended to clarify the grammar of the claim. Support for this amendment may be found throughout the specification.

The specification has been amended to insert a new Sequence Listing.

No new matter is added. As such, the Examiner is requested to enter the above amendments.

In the Office Action dated October 10, 2008, the Examiner objected to the specification because sequence identifiers were not provided for the sequences in Figure 4. In the Response filed February 10, 2009, a replacement drawing for Figure 4 that included sequence identifiers was provided, and a replacement Sequence Listing was submitted to incorporate those sequences into the specification. However, the replacement Sequence Listing was not accompanied by a Certification Regarding Sequence Listing. The Applicants thank the Examiner for pointing out this oversight and provide this Sequence Listing again herein, accompanied by the appropriate amendment to the Specification and Certification, provided immediately below.

**CERTIFICATION REGARDING SEQUENCE LISTING**

I hereby certify that the enclosed Sequence Listing is being submitted under 37 CFR §§ 1.821(c) and (e) in paper (Seqlist_CFR.pdf) and computer readable form (Seqlist_CFR.txt).

As required by 37 CFR 1.821(f), I hereby state that the content of the paper and computer readable copy of the Sequence Listing, submitted in accordance with 37 C.F.R. §1.821(c) and (e) are the same. The Computer Readable Format (CRF), being submitted under 37 CFR §§ 1.52(e) and 1.824, is formatted on IBM-PC, the operating system compatibility is MS-Windows and the file listing is:

Seqlist.txt 47 KB created June 1, 2009.

I hereby certify that the enclosed submission includes no new matter. The Sequence Listing was prepared with the software FASTSEQ, and conforms to the Patent Office guidelines. Applicant respectfully submits that the subject application is in adherence to 37 CFR §§ 1.821-1.825.

**REJECTIONS UNDER §112, ¶1**

Claims 1-8, 12-13, 17 and 27 are rejected under 35 U.S.C. 112, first paragraph because the specification, while being enabling for an isolated nucleic acid molecules selected from the group consisting of a nucleic acid that encodes a protein comprising the amino acid sequence as shown in SEQ ID NOs: 4, 18, 20, 22, 24, 26 or 28 and cells and vectors comprising said nucleic acid, allegedly does not reasonably provide enablement for other claimed embodiments embraced by the breadth of the claims.

With respect to enablement, courts have held that: "[t]he test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." *United States v. Telectronics, Inc.,* 8 USPQ 2d 1217, 1233 (Fed. Cir. 1988), *cert. denied*, 490 U.S. 1046 (1989). See also *Genentech, Inc. v. Novo Nordisk*, 42 USPQ 2d 1001 (Fed. Cir. 1997), *cert. denied*, 522 U.S. 963 (1997); *Scripps Clinic and Research Foundation v. Genentech, Inc.*, 18 USPQ 2d 1001 (Fed. Cir. 1991).

Claim 1 recites "an isolated nucleic acid molecule that encodes a fluorescent protein, wherein the nucleic acid is selected from the group consisting of (a) a nucleic acid that encodes a protein comprising the amino acid sequence as shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28; and (b) a nucleic acid that encodes a protein that has at least about 80% sequence identity to the amino acid sequence of (a) above". Thus, Claim 1 is directed to an isolated nucleic acid molecule that 1) encodes a fluorescent protein, and 2) has at least about 80% sequence identity to SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28.

In making this rejection, the Examiner asserted in the Office Action of October 10, 2008 that "the claims encompass nucleic acids encoding fragments of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28 as well as variants having 80% identity to said SEQ IDs. The level of experimentation to determine which of the fragments or protein variants would encode

or have the desired and useful activity would be undue." (reproduced in the Final Office Action, p. 4, l. 5-8).

In the response filed February 10, 2009, Applicants argued that it would not require an undue amount of experimentation to determine which nucleic acids encoding a protein with 80% homology to the recited SEQ IDs that maintain fluorescent properties.

In response, the Examiner asserted in the Final Office Action dated April 7, 2009 that "a requirement for exhibiting a fluorescent property is not recited in the body of the claims and it is not clear exactly what a 'fluorescent property' would encompass" (Final Office Action, p. 4, l. 20-21). Additionally, the Examiner asserts that "further experimentation is necessary to correlate the structure with the function. Experimentation that requires routine repetition to find something that is already known to exist is not undue. However, experimentation to acquire new knowledge (i.e. map out functional domains of a protein) is undue." (p. 5, l. 3-6)

The Applicants have canceled claim 12, the only claim drawn to fragment of the claimed SEQ IDs. However, the Applicants maintain herein that, in view of the specification and the art, one of ordinary skill in the art would not have to perform an undue amount of experimentation to determine which nucleic acids encoding a protein with 80% homology to the recited SEQ IDs maintain fluorescent properties. Applicants respectfully submit that in making this rejection, the Examiner has focused on the general art of protein structure and function rather than on the more relevant art of protein structure and function as it pertains to fluorescent proteins. As discussed below, the Applicants submit that there exists relevant art that demonstrates a well-developed understanding of the structure of these proteins and how changes to structure impact protein function. As such, the art establishes a precedent for extrapolation without undue experimentation from the examples presented in the pending application to the claimed genus of nucleic acids.

As discussed above, the claims recite "an isolated nucleic acid molecule that encodes a fluorescent protein". Accordingly, contrary to the Examiner's assertions, a requirement for exhibiting a fluorescent property is, in fact, recited in the body of the claims. Furthermore, the Applicants submit that the specification and the art provide clear guidance on what a fluorescent property encompasses. The specification teaches that "Fluorescent proteins or fluoroproteins are proteins that exhibit low, medium or intense fluorescence upon irradiation with light of the

appropriate excitation wavelength. The fluorescent characteristic of these proteins is one that arises from the interaction of two or more amino acid residues of the protein, and not from a single amino acid residue. As such, the fluorescent proteins do not include proteins that exhibit fluorescence only from residues that act by themselves as intrinsic fluors, i.e. tryptophan, tyrosine, and phenylalanine." (p. 1, l. 12-17) Similarly, the art teaches that a fluorescent protein derives its fluorescent property from a fluorophore produced by the interaction of two or more amino acid residues working together; see, for example, the review article, Wilson et al. ((1998) Annu. Rev. Cell. Dev. 14:197-230) (Exhibit A), which teaches that the most common fluorophores are historically derivatives of chemical compounds such as fluorescein, rhodamine, coumarin, luciferin and cyanine. Likewise, both the specification and the art teach the reliance of GFP and Anthozoan fluorescent proteins upon their fluorophore for their fluorescence character (specification, p. 1, l. 25-27; Matz et al. ((1999) Nat Biotech 17:969-973) (Exhibit B). Accordingly, the Applicants submit that one of ordinary skill in the art following the teachings of the specification and the art would understand what a fluorescent property in a protein encompasses, and what protein structure encodes such a property, and thus, what is encompassed by the pending claims.

Furthermore, the Applicants submit that the specification and the art teach a wealth of working examples and guidance that the ordinary skilled artisan would be able use to determine which amino acids are necessary and sufficient in the proteins encoded by the disclosed nucleic acids to maintain fluorescence so as to identify other nucleic acid molecules that 1) encode a fluorescent protein, and 2) have at least about 80% sequence identity to SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28, as recited by the pending claims.

The Applicants submit that the specification teaches a multitude of examples of nucleic acids of the claimed genus. The specification teaches 8 examples of wild type nucleic acid sequences (SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15) that encode fluorescent proteins (SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, and 16) from 5 different species of Copepod (*Pontellina plumata, Labidocer aestiva, Pontella meadi, Poltella mediterranea,* and an unidentified species*)*. Methods of identifying other wild type homologues, including degenerate PCR and BLAST searching, or protein purification, sequencing, and BLAST searching, are well understood in the art, and thus, one of ordinary skill in the art would know how to identify other nucleic acid sequences that encoding wild type fluorescent proteins with at least about 80% similarity to those of the claimed genus. Additionally, the degeneracy of the genetic code is well understood

in the art, and thus, one of ordinary skill in the art would know how to design a multitude of other nucleic acid sequences that would also encode these wild type fluorescent proteins. Accordingly, the Applicants submit that they have provided a reasonable number of examples of wild type nucleic acid sequences that encode fluorescent proteins from a reasonable number of species so as to support the species of wild type nucleic acids encompassed by the pending claimed genus.

Furthermore, the Applicants submit that, in view of the art, they have also provided sufficient guidance and written examples to enable the species of nucleic acids encoding mutants of wild type fluorescent proteins encompassed by the claimed genus. The specification teaches the relevance of GFP and Anthozoan protein structure to the structure of the disclosed proteins (Background; Figure 1). The art teaches a well-studied and highly predictable structure of GFP; see, for example, Yang et al. ((1996) Nat. Biotech 14:1246-51) (Exhibit C) and Ormo et al. ((1996) Science 273:1392-95) (Exhibit D). The art teaches that this structure is conserved amongst all fluorescent proteins and can be used to make predictions as to which amino acids can be substituted in these proteins and how without loss of protein function; see, for example, Matz et al. (Exhibit B). Thus, one of ordinary skill in the art would know from the specification that the proteins encoded by the claimed nucleic acids will have a structure resembling that of the well-studied and highly predictable structure of GFP while maintaining at least 80% identity with the proteins encoded by SEQ ID NOs:2 ,4, 6, 8, 10, 12, 14, or 16, and would know from the art exactly what that structure comprises. For example, the specification and the art teaches the reliance of GFP and Anthozoan proteins upon their fluorophore for their fluorescence character (specification, p. 1, l. 27-31; Matz et al.). Thus, the artisan would understand that the amino acid residues that comprise the fluorophore in the disclosed proteins should be conserved, and would use publicly available web-based software such as Clustal W to align these protein sequences and identify these amino acid residues.

Indeed, in addition to providing 8 examples of wild type proteins that are encoded by nucleic acids of the claimed genus, the specification provides 6 examples of mutant proteins (SEQ ID NOs:18, 20, 22, 24, 26, and 28) that could be aligned with GFP and with the 8 proteins encoded by the nucleic acids of the claimed genus so as to identify all of the residues that should be conserved to maintain fluorescence activity. In fact, the specification provides an example of how to perform such alignments; see Figure 1. The Applicants submit that one of ordinary skill in the art would know how to use the alignment in Figure 1 as well as alignments

with other GFP-like proteins know in the art to identify the amino acids to be conserved, for example, the amino acids comprising the fluorophore, so as to retain the fluorescence character of the protein. More importantly, such alignments as the one in Figure 1 demonstrate to the artisan that only 24 of 234 residues are conserved between this family of proteins and GFP and DsRed; that is, the disclosed proteins share only 10.3% identity with GFP, DsRed and one another (see Exhibit E, which is the alignment of the disclosed proteins to one another and to GFP provided in the specification as Figure 1; asterisks at the top of the alignments have been added to denote conserved residues). Accordingly, the artisan would also recognize from such alignments that strict conservation of most amino acids of these proteins is not required to maintain protein function. Thus, the specification provides sufficient guidance and working examples such that one of ordinary skill in the art would know that a high degree of amino acid substitution could be tolerated by the proteins of this family without loss of fluorescence, and would be able to determine which amino acid substitutions those would be.

In support of this expectation that a high degree of amino acid substitutions in these proteins can be tolerated without losing protein function, the art teaches a plethora of GFP mutations that preserve GFP fluorescence activity. For example, Heim et al. ((1996) Current Biol. 6:178-182) (Exhibit F) teaches six mutants comprising mutations in 10 residues of GFP (Table 1). Siemering et al. ((1996) Current Biol. 6(12):1653-63) (Exhibit G) teaches seven additional mutants (mgfp4, mgfpB, mgfpA, mgfp5, mgfp4 + Y66H, mgfpA + Y66H) comprising mutations in another three residues. Yang et al. ((1998) J Biol Chem 273(14):8212-8216) (Exhibit H) teaches two additional mutants comprising mutations in an additional two residues. The art also teaches a plethora of other fluorescent proteins having minimal identity with GFP. For example, Wiedenmann et al. ((2000) PNAS 97(26):14091-6) (Exhibit I) teaches three fluorescent proteins of *Anemonia sulcata* (asFP499, asFP522, asFP595; see Table 1) that, as a group, share only 12.6% identity to GFP (see Figure 5, "consensus" line). Matz et al. (Exhibit B) teaches six fluorescent proteins from Anthozoans that, as a group, share only 11% identity with GFP (see Figure 1, "cns. All" line), and how the fluorescence activity of GFP and other fluorescent proteins relies upon these conserved residues Bevis et al. ((2002) Nat. Biotechnol 20(1):83-7) (Exhibit J) teaches 7 mutants of one of these Anthozoan proteins, dsRed, (N42H, N42Q, DsRed1, dsRed2, DsRed.T1, DsRed.T3, DsRed.T4; see p. 83, col. 2, para. 3-4, p. 84, Table 1), all of which retain fluorescent activity. Campbell et al. ((2002) PNAS 99(12):7877-82) (Exhibit K) teaches 4 more mutants of dsRed (I125R, dimer2, tdimer2, mRFP1; see paragraph bridging pages 7878-9, and Table 1) that retain fluorescent activity. Shaner et al. ((2004) Nat

Biotechnol 22(12):1567-72) (Exhibit L) teaches a multitude more dsRed-based mutants with improved extinction coefficients, photostability, and a variety of fluorescence spectra (see, for example, Table 1).

Thus, the artisan would find a wealth of guidance and working examples in the specification and the art which teach that the proteins encoded by the claimed nucleic acids can tolerate a high degree of amino acid substitution while still retaining fluorescence activity. Furthermore, the artisan would recognize that they could use alignments of the proteins provided in the specification with those provided in the art to identify exactly which amino acids of the proteins encoded by the claimed nucleic acids are conserved and should not be mutated versus those which are not conserved and could be mutated so as to retain fluorescence activity. Moreover, the specification teaches methods of testing these predictions, by teaching methods of making mutant nucleic acids encoding mutant proteins (p. 6, l. 21-p. 7, l. 4; see also p. 20, l. 20-31), and of testing these mutant nucleic acid, for example by transfecting the nucleic acids into cells in culture, waiting 24 hours, and imaging the cells on a fluorescence microscope (p. 23, l. 9-18). These methods are considered well-developed and routine in the art, as they rely upon the highly predictable arts of DNA manipulation, nucleic acid expression, protein characterization, and microscopy. Thus, in view of the well developed and highly predictable art as discussed above, and contrary to the Examiner's assertions, what further experimentation that may be required of the ordinary skilled artisan to practice the claimed invention is, in fact, "routine repetition to find something that is already known to exist", and thus is not undue.

Therefore, the Applicants maintain that the specification in view of the art provides a reasonable amount of guidance and working examples on the relationship between the structure of the disclosed proteins and their function as fluorescent molecules that one of ordinary skill in the art would be able to identify other species of the claimed genus without undue experimentation.

In view of these remarks, reconsideration and withdrawal of the rejection is requested.

## CONCLUSION

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number EURE-005.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: June 8, 2009

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Enclosure(s): Seqlist.txt
Seqlist.pdf
Exhibits A-L.pdf, which includes the following:
- Exhibit A_Wilson et al. ((1998) Annu. Rev. Cell. Dev. 14:197-230)
- Exhibit B_Matz et al. ((1999) Nat Biotech 17:969-973)
- Exhibit C_Yang et al. ((1996) Nat. Biotech 14:1246-51)
- Exhibit D_Ormo et al. ((1996) Science 273:1392-95)
- Exhibit E_Figure 1 alignment
- Exhibit F_Heim et al. ((1996) Current Biol. 6:178-182)
- Exhibit G_Siemering et al. ((1996) Current Biol. 6(12):1653-63)
- Exhibit H_Yang et al. ((1998) J Biol Chem 273(14):8212-8216)
- Exhibit I_Wiedenmann et al. ((2000) PNAS 97(26):14091-6)
- Exhibit J_Bevis et al. ((2002) Nat. Biotechnol 20(1):83-7)
- Exhibit K_Campbell et al. ((2002) PNAS 99(12):7877-82)
- Exhibit L_Shaner et al. ((2004) Nat Biotechnol 22(12):1567-72)

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